bio-protocol Preprint

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Conjunction of DNA and primary antibody

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An abbreviated version of this protocol was published in Science Advances in Jan 2022

Computer-aided design of reversible hybridization chain reaction (CAD-HCR) enables multiplexed single-cell spatial proteomics imaging

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Detailed protocol

Dear Noorsher Ahmed,

The following is the detailed protocal for the conjunction of DNA and primary antibody:

1.100K MWCO Amicon Ultra 0.5 mL filter was firstly rinsed with reaction buffer, and centrifuged at 14,000 g for 10 min.
2.After removing the reaction buffer, the filter can be used to purify commercial primary antibodies (PA).
3.PA was mixed with reaction buffer to a volume of 0.5 mL, and then centrifuged at 14,000 g for 10 min.
4.Then, filtrate was removed and the PA was recovered to 0.5 mL with reaction buffer.
5.Repeat step 3-4 at least three times. After the last centrifugation, PA was collected.
6.The collected PA was quantified with nanodrop and suspended in reaction buffer [55 mM sodium phosphate, 150 mM sodium chloride, and 20 mM EDTA (pH 7.2)] to make 1 mg/mL solutions, followed by a 2-hour incubation of 80-fold excess of sulfo-SMCC (dissolved in anhydrous DMSO) at RT to activate the amino group of primary antibodies.
7.A 10K MWCO Zeba spin desalting column was used to separate out redundant sulfo-SMCC by centrifuge ultrafiltration for three rounds.
8.In parallel, we reduced the thiol-modified initiators with DTT at a molar ratio of 1:500 in the reaction buffer for 1 hour at 37°C. Excess DTT in the thiolated DNA solution was removed by a 3K MWCO Zeba spin desalting column.
9.The sulfo-SMCC–activated primary antibodies were mixed with a threefold molar excess of reduced initiators and reacted at RT for 2 hours.
10.Lastly, protein-DNA conjugates were further purified by the 10K MWCO Zeba Spin Desalting Column and stored at 4°C for short-term usage or at −20°C until used.

As an alternative, a strategy based on the NHS-PEG4-N3 cross-linking agent is another option (Protein spherical nucleic acids, Chad A. Mirkin).

Best wishes!

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Liu, X. , Sun, F. , Yang, Y. , Zhu, X. and Tan, W. (2022). Conjunction of DNA and primary antibody. Bio-protocol Preprint. bio-protocol.org/prep1553.
2. Liu, X., Mao, D., Song, Y., Zhu, L., Isak, A. N., Lu, C., Deng, G., Chen, F., Sun, F., Yang, Y., Zhu, X. and Tan, W.(2022). Computer-aided design of reversible hybridization chain reaction (CAD-HCR) enables multiplexed single-cell spatial proteomics imaging. Science Advances 8(2). DOI: 10.1126/sciadv.abk0133

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